Mesopotamia J. of Agric. ISSN: 2224 - 9796 (Online) مـجلــة زراعــة الـرافديـن
Vol. (46) No. (1) 2018 ISSN: 1815 - 316 X (Print) المجلد (46) العدد (1) 2018

IDENTIFICATION AND BIOLOGICAL CONTROL OF BASAL STEM AND ROOT ROT DISEASE OF SNAPDRAGON

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ABSTRACT

For the first time wilt symptoms have been detected on the Snapdragon (*Antirrhinum majus* L) plants cultivated in the parks of Mosul University. The isolation of fungi from wilted plants on PDA medium revealed the presence of seven genera including; *Alternaria, Aspergillus, Cladosporium, Fusarium, Macrophomina, Penicillium* and *Stemphyllium. Fusarium oxysporum* was the most frequent fungi approached 36.0 x 10^2 Colony Forming Unit/gm soil. *F.oxysporum* reduced significantly the chlorophyll content of infected plants, and the percentage of its inhibition increased up to 81.88 in severe infections. The infection caused significance stunting of plants when plant height inhibited by 58.09% in dead plants. Antagonism ability of *Trichoderma* species as *T.harzianum, T. viride,* and *T. reesei* against *F. oxysporum* showed control efficiency of.1.666, 2.333, and 2.666 respectively.

Key word: Snapdragon (*Antirrhinum majus*), *Fusarium, Trichoderma.*

Received: 3/11/2013, Accepted: 17/2/2014.

INTRODUCTION

Snapdragon (*Antirrhinum majus* L.) plants, well-known species cultivated in parks of Mosul University during in winter and flourishes in spring. The soil borne fungi can infect these plants (Greer, 2002).It has been reported that, wilt symptoms caused death for half of these plants and with reduction their flowers up to 67% compared to that plants produced in solarization or by Methyl bromide evaporated or Metam sodium (McSorley,*et al*, 2004).

The symptoms of the disease are influenced by air or soil temperature. and were light under the temperature 24-32^{O}C. However, these might be appeared in some new plants which grown later on as the temperature slightly elevated (Alfeieri,*et al*, 1994).

Snapdragon's roots were utilized to produce oil, which is slightly less effective than olive oil. Its leaves and flowers were used in medicine as stimulant and also for treatment of ulcers and infections. Snapdragon with its different colures as green and golden were used as natural coloring material for food industry in some countries.

The aim of this study was to find the causal fungi responsible for the infection with basal stem and root rot disease of snapdragon plants, and to find the biological control agent.

MATERIALS AND METHODS

Isolation of fungi :A number of Snapdragons (*Antirrhinum majus* L) plants with wilt symptoms were collected from parks scattered in University of Mosul. In the laboratory they were washed by tap water for 10 minutes to remove soil and debris. Portions from roots were cut off into small pieces (0.5-1.0 cm) and treated with 1% sodium hypochlorite for 3 minutes, washed with sterilized distilled water and dry with sterilized filter paper. The surface sterilized samples were transferred to a plastic Petri dish (diameter 9cm) containing 15 ml Potato Dextrose Agar (PDA) supplemented with 100 ppm of streptomycin , 4 pieces of the root / Petri dish. Specimens were incubated at 25^{O}C for 1 week. Some culture was diagnosed microscopically according to approved classifications keys (Booth,1971; Barnett and Hunter,

2006; Pitt and Hocking,2009).They were purified then transferred to test tube containing 10 ml of slant PDA and kept in the refrigerator until use.

The isolates of *Fusarium* were transferred to Petri dishes containing 10 ml carnation leaf agar (Nelson,*et al*,1983) to enhance their sporulation. Fungal isolates were left for 7 days in the incubator under 25 $\pm 2^{\circ}$ C under successive 12 hr. light / 12 hr. darkness.

Preparation of sporulation suspension: Each plastic Petri dishes (9.0 cm diam.) contained 10 ml of PDA were inoculated with the *F.oxysporum* and incubated at 25°C .After a week of incubation, 10 ml of sterilized distilled water was added to each dish to obtain sporulation suspension .The concentration of this suspension was 5 x 10^4 spore/ml determined by using haemocytomerter.

Pathogenicity: Seeds of Snapdragons plants were cultivated in wooden box (40x40x10cm) contains 1% formalin sterilized soil (Mustafee and Cattopadhyag, 1981) After 20 days of cultivation, the Snapdragon seedling produced had to 2-4 leaves were transferred to other pots (15 cm in diameter) containing 2 kg of sterilized soil in at ratio seedling / pot. After 10 days the sides of the roots were cut off by sterilized surgical scalpel for allowing the fungi to pass through the roots. The spore suspension of 25 ml was added per pot for each fungus (Saydam, *et.al*, 1973). Others pots were left with only sterilized distilled water for comparison (24 pots, 7 fungi for comparison). All pots were kept in glasshouse condition (Department of Biology, College of Science, University of Mosul) for one month for observation and data regular (changes, symptoms, re-isolation from infected plants).

Seedlings of Snapdragon with 2-4 leaves were transferred to pots (15 cm in diameter) containing 2 kg of sterilized soil. After 10 days and by using sterilized surgical scalpel, the sides of roots were cut off to allow the invasion of *F. oxysporum* (Wang, *et.al*, 2007). Soil of five pots with sterilized distilled water were left for comparison. All pots were kept in glasshouse condition until flowering. Longitudinal sections were prepared from stems base and infected roots to identify the infected tissue and to estimate chlorophyll percentage by chlorophyll-meter (S pad-502; Konica Minolta-Japan). The results were statistically analyzed using Duncan's multiple range test (Anter, 2010)

Biological control of *Fusarium*: Three species of *Trichoderma* were used for biological control program. *T. reesei* was dispatched from Dr. Joan M. Kelly, University of Adelaide, Australia), while the other fungi *T. harzianum* and *T. viride* were supplied from Agriculture college of Mosul University.

The program used the Dual culture technique of *Trichoderma* with *F. oxysporum* (isolated from infected Snapdragon) on 10 ml of PDA in Petri dishes (9 cm in diameter) supplemented with 50 mg / l streptomycin 50 mg / l. The medium in each dish was separated into two equal parts, one part was inoculated with 0.5 cm of biological agent and the other part inoculated with a similar disc of *F. oxysporum* (one week old). The distance between these two discs was approximately 4 cm. For comparison, one was inoculated with bio-resistant fungi and the other with pathogenic fungi.

The experiment was replicated three times for each fungus and the data were reported after one week of incubation dish under temperature of 25$\pm$ 2 ^{O}C. The inhibition degree was calculated according to 5 standard divisions (Bell *et al*, 1982). Results were analyzed by Duncan's multiple range test (Anter, 2010).

RESULTS AND DISCUSSION

Isolation of fungi: Seven genera of fungi namely, *Alternaria; Aspergillus; Cladosporium; Fusarium; Macrophomin; Penicillium* and *Stemphyllium* were isolated .They were cultivated on PDA medium from infected legs and roots of Snapdragon. The most widely available was *F.oxysporum* followed by *A. alternate* (Table,1). Nelson,*et.al.*(1983) reported the isolation of many *Fusarium spp*. from Snapdragon including isolates of *F. oxysporum* which can infect various plants. Wang, *et.al*. (2007) found that isolates of *F. oxysporum* isolated from Snapdragon have the ability to infect plants with basal stem and root rot disease.

Pathological activity: The present results revealed that the plants infected with fungi showed symptoms of withered and yellowish leaves. The yellowish symptoms developed firstly on downward leaves and then going upwards, and some time found on one side only.

These symptoms appeared from root going upward leading to withered, yellowish and death of leaves (Fig.1.A).The longitudinal sections of stems showed brown or dark brown colored vessels (Fig.1.B), due to reisolated *F. oxysporum* was noticed from infected plants.

Table (1): Isolated fungi from basal stem and root rot of infected Snapdragon (*Antirrhinum majus* L.) plants.

Fungi	Frequency (%)
Alternaria alternata	59.0
Aspergillus niger	5.0
Cladosporium spp.	21.0
Fusarium oxysporum	75.0
Macrophomina phaseolina	5.0
Penicillium spp.	6.0
Stemphylium harbarum	20.0

The species of fungi cultivated on PDA medium which isolated from soil surrounding the Basal stem (roots) of infected Snapdragon are recorded in Table (2). Data showed that the most isolated fungus was *F. oxysporum* approaching $36x10^2$ CFU / gm soil, followed by *Alternaria alternata*. Other fungi showed no effect. This might be due to the availability of fungi in soil as well as on parts of infected plants which may be lost during the process of explants cleaning and sterilization.

Table (2): Fungi cultivated on PDA medium which isolated from basal stem and root rot of Snapdragon (*Antirrhinum majus* L) plants.

Fungi	10^2 CFU / gm soil
Alternaria alternata	22.0
Cladosporium spp.	8.0
Fusarium oxysporum	36.0
Stemphyllium harbarum	5.0

A B

Figure (1): Snapdragon plants (*Antirrhinum majus* L) infected with *F. oxysporum*.
A- Wilt symptoms on mature plants.
B- Longitudinal section at base stem discoloration appears carrier vessels (arrow).

It has also been found that infection with *F. oxysporum* decreased significantly of chlorophyll percentage in infected plants when comprised with health ones, even in light infection. This leading to increase the percent of inhibition up to 81.88% occurred in heavily infected plants (Table, 3). Infection caused plants shorting while the inhibition rate reaches 58.09% in dead plants (Table,3). However, Malek, *et.al*. (2005) reported that chlorophyll content might be related to certain circumstances including stress of many plant spp. as well as infection is considered as biological stress, furthermore the etiology of plant pathology might alter photosynthesis process in plants (Susaki and Ode, 1994). Also,the results in table(3) indicate that virulent infection reduced plants height

Table (3): Chlorophyll content and height of Snapdragon (*Antirrhinum majus* L) plants infected with *F. oxysporum* .

Degree of infection	chlorophyll (%)	Inhibition (%)	Plant height (cm)	Inhibition (%)
Healthy plants	* 46.2 a	0.0	84.2 a	0.0
Light infected Plants	38.77 b	16.08	64.7 b	23.15
Moderate infected Plants	19.12 c	58.16	59.0 b	29.92
Heavy infected Plants	8.37 d	81.88	40.1 c	52.37
Dead plants	4.75 d	100	20.2 d	58.09

*average of five replication.
Numbers with similar letters has no significantly differences in 5% level according to Duncan test.

Biological control of *Fusarium*: The results indicated that all three species of *Trichoderma* were highly efficient against *F.oxysporum* which caused disease of Snapdragon (Table, 4). There is no difference with *T. harzianum* with its effectiveness of 1.666 while *T. viride* and *T. reesei* showed effectiveness of 2.333 and 2.666 respectively.

Trichoderma was used extensively in various programs of biological control of plant pathogens. Many species of these fungi showed their ability to infect plants including *F.oxysporum* (Sivan and Chet, 1986). Charaborty and Chatterjee (2008) studied the biological control of *Fusarium* wilt of *Solanum melongena* by *Trichoderma spp*. They used five species including *T. harzianum*, *T. viride*, *T. lignorum*, T. *hamatum*, and *T. reesei*. *T.harzianum* showed high inhibition rate of 86%. They also indicated that the use of both *T. harzianum* and *T. viride* reduced the frequency and spread of *Fusarium saloni* in soil under field conditions .This is might be due to the production of siderophores by biological agent with enzymes decomposition of cell wall chitinase and B-1,3-glucanase.

El-Farnawany (1996) reported that resistant is due to direct penetration of the infected fungal hyphae or through establishment of appresorium forming nodules on the hyphae of pathogenic fungi, or it may be due to one or more antibiotics which were released to the environment such as Trichodermin, Emodine, Glyatoxins or Pachybascine, that inhibit growth of pathogenic fungi (Papavizas and Lewis, 1989).

Table (4): The ability of three species of *Trichoderma* against *F. oxysporum* causing wilt symptoms of *Antirrhinum majus* plants.

Species	Degree of antagonism
T. harzianum	1.666 *
T. viride	2.333
T. reesei	2.666

*average of three replicates .
Numbers with similar letters has no significantly differences in 5% level according to Duncan test.

ACKNOWLEDGMENT: I would like to express my sincere gratitude and appreciation to Dr. Joan M. Kelly, School of Molecular and Biomedical Science, University of Adelaide, Australia, for her generosity and helpfulness.

مرض اسوداد قاعدة الساق و تعفن جذور نباتات حنك السبع، تشخيص الفطر المسبب ومكافحته حيوياً

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الخلاصة

لأول مرة تسجل علامات الذبول على نباتات حنك السبع (*Antirrhium majus* L) النامية في حدائق جامعة الموصل. واظهر عزل الفطريات، من التربة المحيطة بجذور النباتات التي تعاني من الذبول، على وسط PDA الكشف عن سبعة من الأجناس الفطرية : *Alternaria* و*Aspergillus* و*Cladosprium* و *Fusarium* و *Macrophomina* و *Penicillium* و *Stemphyllium*. وكان فطر *F.oxysporum* الأكثر تكرارا في تواجده و سجل عدد الوحدات المكونة لمستعمراته $10X36.0^2$ مستعمرة / غم تربة. أظهرت نتائج الدراسة الحالية أن الإصابة بالفطر *F.oxysporum* تسبب اختزالا معنويا واضحا في نسبة كلوروفيل النباتات المصابة ولوحظ زيادة اختزال نسبته مع شدة الإصابة (81.88 %) في نباتات حنك السبع. كما عانت هذه النباتات من تقزم بصورة متماثلة وقد وصلت نسبة تثبيط ارتفاع النباتات إلى 58.09%. من النتائج المرغوبة في هذا البحث أن المقاوم الحيوي *Trichoderma* بأنواعه *T.harzianum* و*T.viride* و *T.* reesei أبدت قدرة تضادية تجاه فطر *F.oxysporum* محققه 1.666 و2.333 و2.666 على التوالي دون ملاحظة فروق معنوية بينها.

الكلمات الدالة: نباتات حنك السبع، فطريات *Fusarium* ، *Trichoderma*

تاريخ تسلم البحث: 2013/11/3، وقبوله: 2014/2/17.

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